

WEST Search History

DATE: Thursday, August 08, 2002

Set Name Query

side by side

Hit Count Set Name

result set

DB=USPT; PLUR=YES; OP=ADJ

L9	sin-yoke-min.in.	0	L9
L8	gong-zhiyuan.in.	1	L8
L7	L6 and vaccin?\$4	4	L7
L6	fish and white spot disease	8	L6
L5	L1 and (i-antigen or immobilization)	2	L5
L4	l1 and recombinant	2	L4
L3	l1 and L2	2	L3
L2	white spot disease	8	L2
L1	ichthyophthirius multifiliis	16	L1

END OF SEARCH HISTORY

9/196161
11/01

=> e sin min yoke/au

E1	1	SIN MIN CHOUL/AU
E2	1	SIN MIN CHUOL/AU
E3	0 -->	SIN MIN YOKE/AU
E4	1	SIN MING CHIEN/AU
E5	1	SIN MING LAM/AU
E6	1	SIN MINGKUN/AU
E7	1	SIN MUN GIL/AU
E8	1	SIN MYONG HYON/AU
E9	1	SIN MYUNG KYUN/AU
E10	19	SIN N/AU
E11	2	SIN N C/AU
E12	2	SIN N D NIK/AU

=> e min sin yoke/au

E1	1	MIN SIN Y/AU
E2	1	MIN SIN YAKE/AU
E3	3 -->	MIN SIN YOKE/AU
E4	1	MIN SIOW W/AU
E5	9	MIN SO YOUN/AU
E6	3	MIN SO YOUNG/AU
E7	1	MIN SOEK R/AU
E8	1	MIN SOHN JUNG/AU
E9	1	MIN SOHN K/AU
E10	3	MIN SOK KI/AU
E11	2	MIN SON HO/AU
E12	1	MIN SON KI/AU

=> s e1-e3

L27 5 ("MIN SIN Y"/AU OR "MIN SIN YAKE"/AU OR "MIN SIN YOKE"/AU)

=> e jin lam toong/au

E1	2	JIN LAIZHE/AU
E2	1	JIN LAM T/AU
E3	1 -->	JIN LAM TOONG/AU
E4	65	JIN LAN/AU
E5	1	JIN LAN D/AU
E6	1	JIN LAN HE/AU
E7	1	JIN LAN L/AU
E8	1	JIN LAN XU SHUHUAI/AU
E9	1	JIN LAN ZHANG SHUYANG/AU
E10	2	JIN LANFEN/AU
E11	1	JIN LANFU/AU
E12	1	JIN LANPING/AU

=> s e2-e3

L28 2 ("JIN LAM T"/AU OR "JIN LAM TOONG"/AU)

=> e lam toong jin/au

E1	6	LAM TONY Y K/AU
E2	2	LAM TOONG J/AU
E3	20 -->	LAM TOONG JIN/AU
E4	4	LAM TRANG/AU
E5	2	LAM TRANG T/AU
E6	1	LAM TRANH H/AU
E7	1	LAM TRI T/AU
E8	1	LAM TRIEU IAN/AU
E9	2	LAM TRIEU LAN/AU
E10	2	LAM TSAN CHIH LI/AU
E11	1	LAM TSANG SING/AU
E12	1	LAM TSE FUN/AU

=> s e2-e3

L29 22 ("LAM TOONG J"/AU OR "LAM TOONG JIN"/AU)

=> e gong zhiyuan/au

E1	1	GONG ZHIYI HUANG YUGUANG/AU
E2	2	GONG ZHIYONG/AU
E3	108 -->	GONG ZHIYUAN/AU
E4	1	GONG ZHIYUN/AU
E5	12	GONG ZHIZHONG/AU
E6	1	GONG ZHIZONG/AU
E7	1	GONG ZHODI/AU
E8	2	GONG ZHONG MING/AU
E9	1	GONG ZHONG WEI/AU
E10	3	GONG ZHONGGUI/AU
E11	1	GONG ZHONGHUA/AU
E12	1	GONG ZHONGLIANG/AU

=> s e2-e4

L30 111 ("GONG ZHIYONG"/AU OR "GONG ZHIYUAN"/AU OR "GONG ZHIYUN"/AU)

=> s l27-l30

3 FILES SEARCHED...

L31 130 (L27 OR L28 OR L29 OR L30)

=> s l31 and ichthyophthirius

L32 3 L31 AND ICHTHYOPHTHIRIUS

=> d bib ab 1-3

L32 ANSWER 1 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS

AN 1998:78040 BIOSIS

DN PREV199800078040

TI Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine.

AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong

Jin; Sin, Yoke Min (1)
 CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge
 Crescent,
 Singapore 119260 Singapore
 SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
 ISSN: 0044-8486.
 DT Article
 LA English
 AB A 316 bp gene fragment containing a potential antigenic epitope
 of the 48
 kDa immobilization antigen of *Ichthyophthirius multifiliis*
 (i-AgI) was assembled from six synthetic oligonucleotides and
 cloned into
 a bacterial expression vector pGEX2T. The gene construct was
 introduced
 into *Escherichia coli* and the glutathione S-transferase-iAgI
 (GST-iAgI)
 fusion protein was successfully expressed. Antisera against
 GST-iAgI
 fusion protein from catfish showed a positive reaction with a
 tomites
 protein of about 48 kDa, suggesting that the recombinant protein
 contains
 an antigenic epitope of i-AgI. Naive goldfish that were
 immunized with
 purified GST-iAgI fusion protein were challenged with a lethal
 dose of
 infectious tomites of *I. multifiliis*. The results showed that
 the average
 survival rate of the immunized fish was 95% as compared to 55%
 for the
 control fish. All these findings suggest that the recombinant
 GST-iAgI
 fusion protein can be used as a potential vaccine against the
 infection of
I. multifiliis.

L32 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2001 ACS
 AN 1997:781004 CAPLUS
 DN 128:126832
 TI Protection of goldfish against *Ichthyophthirius multifiliis* by
 immunization with a recombinant vaccine
 AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gong, Zhiyuan; Jin
 Lam, Toong; Min Sin, Yoke
 CS 10 Kent Ridge Crescent, School of Biological Sciences, National
 University
 of Singapore, Singapore, 119260, Singapore
 SO Aquaculture (1997), 158(1,2), 1-10
 CODEN: AQCLAL; ISSN: 0044-8486
 PB Elsevier Science B.V.
 DT Journal
 LA English
 AB A 316 bp gene fragment contg. a potential antigenic epitope of
 the 48 kDa
 immobilization antigen of *I. multifiliis* (i-AgI) was assembled
 from 6
 synthetic oligonucleotides and cloned into a bacterial
 expression vector
 pGEX2T. The gene construct was introduced into *Escherichia coli*
 and the

glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a pos. reaction with a tomite protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive goldfish that were immunized with purified GST-iAgI fusion protein were challenged with a LD of infectious tomite of *I. multifiliis*. The results showed that the av. survival rate of the immunized fish was 95% as compared to 55% for the control fish. Apparently, the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of *I. multifiliis*.

L32 ANSWER 3 OF 3 LIFESCI COPYRIGHT 2001 CSA

AN 1998:26672 LIFESCI

TI Protection of goldfish against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine

AU He, J.; Yin, Z.; Xu, G.; Gong, Z.; Jin Lam, T.; Min Sin, Y.

CS School of Biological Sciences, National University of Singapore, 10 Kent

Ridge Crescent, Singapore 119260, Singapore

SO AQUACULTURE, (1997:1201) vol. 158, no. 1-2, pp. 1-10.

ISSN: 0044-8486.

DT Journal

FS Q4

LA English

SL English

AB A 316 bp gene fragment containing a potential antigenic epitope of the 48

kDa immobilization antigen of *Ichthyophthirius multifiliis* (i-AgI) was assembled from six synthetic oligonucleotides and cloned into

a bacterial expression vector pGEX2T. The gene construct was introduced

into *Escherichia coli* and the glutathione S-transferase-iAgI (GST-iAgI)

fusion protein was successfully expressed. Antisera against GST-iAgI

fusion protein from catfish showed a positive reaction with a tomite

protein of about 48 kDa, suggesting that the recombinant protein contains

an antigenic epitope of i-AgI. Naive goldfish that were immunized with

purified GST-iAgI fusion protein were challenged with a lethal dose of

infectious tomite of *I. multifiliis*. The results showed that the average

survival rate of the immunized fish was 95% as compared to 55% for the

control fish. All these findings suggest that the recombinant GST-iAgI

fusion protein can be used as a potential vaccine against the infection of

I. multifiliis.

=> s l31 and goldfish

L33 5 L31 AND GOLDFISH

=> d bib ab 1-5

L33 ANSWER 1 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS

AN 1998:78040 BIOSIS

DN PREV199800078040

TI Protection of **goldfish** against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine.

AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong Jin; Sin, Yoke Min (1)

CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent,

Singapore 119260 Singapore

SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.

ISSN: 0044-8486.

DT Article

LA English

AB A 316 bp gene fragment containing a potential antigenic epitope of the 48

kDa immobilization antigen of *Ichthyophthirius multifiliis*

(i-AgI) was

assembled from six synthetic oligonucleotides and cloned into a bacterial

expression vector pGEX2T. The gene construct was introduced into *Escherichia coli* and the glutathione S-transferase-iAgI

(GST-iAgI) fusion

protein was successfully expressed. Antisera against GST-iAgI fusion

protein from catfish showed a positive reaction with a tomite protein of

about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive **goldfish** that were immunized with purified GST-iAgI fusion protein were challenged with a

lethal dose

of infectious tomite of *I. multifiliis*. The results showed that the

average survival rate of the immunized fish was 95% as compared to 55% for

the control fish. All these findings suggest that the recombinant GST-iAgI

fusion protein can be used as a potential vaccine against the infection of

I. multifiliis.

L33 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2001 BIOSIS

AN 1995:264501 BIOSIS

DN PREV199598278801

TI Expression of the antifreeze protein gene in transgenic **goldfish** (*Carassius auratus*) and its implication in cold adaptation.

AU Wang, Renxue; Zhang, Peijun; Gong, Zhiyuan; Hew, Choy L. (1)

CS (1) Dep. Clin. Biochem., Univ. Toronto, 100 College St., Room
351,
Toronto, ON M5G 1L5 Canada
SO Molecular Marine Biology and Biotechnology, (1995) Vol. 4, No.
1, pp.
20-26.
ISSN: 1053-6426.
DT Article
LA English
AB Ocean pout (*Macrozoarces americanus*) antifreeze protein (AFP)
genes (
apprx 10⁻⁶ copies) were microinjected into the oocytes of
goldfish
(*Carissius auratus*). Out of a total of 303 oocytes injected, 235
(77.6%)
were normally fertilized after in vitro maturation and
insemination, and
136 (57.9%) of the fertilized eggs were hatched. Dot blot
analysis of
genomic DNA from 2-month-old fish indicated that 30 of the fish
(22%) were
positive for the AFP transgene. The numbers of integrated AFP
genes ranged
from a single copy to multiple copies per cell. Two P-1 male
founders, Y45
and GV16, were crossed separately with a control female, and the
inheritance of the AFP transgene was analyzed. Results from
Southern
blotting and polymerase chain reaction were found to be 7% (5
out of 82)
and 58.8% (20 out of 34), respectively, indicating that these
two founders
are mosaic. The percentage of positive F-1 generated from Y45F,
crossed
with a control female was 56%, consistent with a Mendelian
inheritance.
Mature AFP detected by immunoblotting was expressed in both F-1
and F-2
offspring. These studies indicate that the antifreeze protein
gene is
successfully transferred and expressed in **goldfish**. Transgenic
goldfish are significantly more cold tolerant than controls when
challenged with low temperatures. These studies suggest that the
AFP gene
may have application in providing cold tolerance in addition to
freeze
resistance for a variety of fish species.

L33 ANSWER 3 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 1997:781004 CAPLUS
DN 128:126832
TI Protection of **goldfish** against *Ichthyophthirius multifiliis* by
immunization with a recombinant vaccine
AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gong, Zhiyuan; Jin
Lam, Toong; Min Sin, Yoke
CS 10 Kent Ridge Crescent, School of Biological Sciences, National
University
of Singapore, Singapore, 119260, Singapore
SO Aquaculture (1997), 158(1,2), 1-10

CODEN: AQCLAL; ISSN: 0044-8486
PB Elsevier Science B.V.
DT Journal
LA English
AB A 316 bp gene fragment contg. a potential antigenic epitope of the 48 kDa immobilization antigen of *I. multifiliis* (i-AgI) was assembled from 6 synthetic oligonucleotides and cloned into a bacterial expression vector pGEX2T. The gene construct was introduced into *Escherichia coli* and the glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a pos. reaction with a tomite protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive **goldfish** that were immunized with purified GST-iAgI fusion protein were challenged with a LD of infectious tomite of *I. multifiliis*. The results showed that the av. survival rate of the immunized fish was 95% as compared to 55% for the control fish. Apparently, the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of *I. multifiliis*.

L33 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2001 ACS
AN 1995:686431 CAPLUS
DN 123:80027
TI Expression of the antifreeze protein gene in transgenic **goldfish** (*Carassius auratus*) and its implication in cold adaptation
AU Wang, Renxue; Zhang, Peijun; **Gong, Zhiyuan**; Hew, Choy L.
CS Inst. Oceanology, Academia Sinica, Shandong, Peop. Rep. China
SO Mol. Mar. Biol. Biotechnol. (1995), 4(1), 20-6
CODEN: MMBBEQ; ISSN: 1053-6426
DT Journal
LA English
AB Ocean pout (*Macrozoarces americanus*) antifreeze protein (AFP) genes (.apprx.106 copies) were microinjected into the oocytes of **goldfish** (*Carassius auratus*). Out of 303 oocytes injected, 235 (77.6%) were normally fertilized after in vitro maturation and insemination, and 136 (57.9%) of the fertilized eggs were hatched. Dot blot anal. of genomic DNA from 2-mo-old fish indicated that 30 of the fish (22%) were pos. for the AFP transgene. The nos. of integrated AFP genes ranged from a single copy to multiple copies per cell. Two P1 male founders, Y45 and GV16, were crossed sep. with a control female, and the inheritance of the AFP transgene was analyzed. Results from Southern

blotting and PCR were 7% (5 out of 82) and 58.8% (10 out of 34), resp., indicating that these 2 founders are mosaic. The percentage of pos. F2 generated from Y45F1 crossed with a control female was 56%, consistent with a Mendelian inheritance. Mature AFP detected by immunoblotting was expressed in both F1 and F2 offspring. These studies indicate that the antifreeze protein gene is successfully transferred and expressed in **goldfish**. Transgenic **goldfish** are significantly more cold tolerant than controls when challenged with low temps. These studies suggest that the AFP gene may have application in providing cold tolerance in addn. to freeze resistance for a variety of fish species.

L33 ANSWER 5 OF 5 LIFESCI COPYRIGHT 2001 CSA

AN 1998:26672 LIFESCI

TI Protection of **goldfish** against *Ichthyophthirius multifiliis* by immunization with a recombinant vaccine

AU He, J.; Yin, Z.; Xu, G.; Gong, Z.; Jin Lam, T.; Min Sin, Y.

CS School of Biological Sciences, National University of Singapore, 10 Kent

Ridge Crescent, Singapore 119260, Singapore

SO AQUACULTURE, (1997) 1201 vol. 158, no. 1-2, pp. 1-10.

ISSN: 0044-8486.

DT Journal

FS Q4

LA English

SL English

AB A 316 bp gene fragment containing a potential antigenic epitope of the 48

kDa immobilization antigen of *Ichthyophthirius multifiliis* (i-AgI) was

assembled from six synthetic oligonucleotides and cloned into a bacterial

expression vector pGEX2T. The gene construct was introduced into *Escherichia coli* and the glutathione S-transferase-iAgI

(GST-iAgI) fusion

protein was successfully expressed. Antisera against GST-iAgI fusion

protein from catfish showed a positive reaction with a tomite protein of

about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive **goldfish** that were immunized with purified GST-iAgI fusion protein were challenged with a lethal dose

of infectious tomite of *I. multifiliis*. The results showed that the

average survival rate of the immunized fish was 95% as compared to 55% for

the control fish. All these findings suggest that the recombinant GST-iAgI

fusion protein can be used as a potential vaccine against the infection of

I. multifiliis.

=> s l31 and vaccin?

L34 7 L31 AND VACCIN?

=> dup rem l34

PROCESSING COMPLETED FOR L34

L35 3 DUP REM L34. (4 DUPLICATES REMOVED)

=> d bib ab 1-3

L35 ANSWER 1 OF 3 USPATFULL

AN 2001:185451 USPATFULL

TI Intracellular antifreeze polypeptides and nucleic acids

IN Hew, Choy, Thornhill, Canada

Gong, Zhiyuan, Toronto, Canada

PA HSC Research and Development Ltd. Partnership, Toronto, Canada
(non-U.S.

corporation)

PI US 6307020 B1 20011023

WO 9728260 19970807

AI US 1998-117121 19981120 (9)

WO 1997-CA62 19970130

19981120 PCT 371 date

19981120 PCT 102(e) date

DT Utility

FS GRANTED

EXNAM Primary Examiner: Carlson, Karen Cochrane; Assistant Examiner:
Robinson,

Hope A.

LREP Townsend and Townsend and Crew LLP

CLMN Number of Claims: 14

ECL Exemplary Claim: 1

DRWN 21 Drawing Figure(s); 20 Drawing Page(s)

LN.CNT 2175

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A family of related intracellular skin type antifreeze
polypeptides and

corresponding coding nucleic acids are provided. These are the
first

skin type intracellular antifreeze polypeptides and coding
nucleic acids

ever reported. The polypeptides are naturally expressed in the
skin of

Winter Flounder, and skin specific promoters are also
provided. The

polypeptides are used to make cells cold-resistant, and to
improve the

palatability of cold foods and liquids. Cold resistant
eukaryotes and

prokaryotes, including plants, animals and bacteria are made
using the

skin-type intracellular antifreeze polypeptides and nucleic
acids.

L35 ANSWER 2 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 1
 AN 2001:334915 BIOSIS
 DN PREV200100334915
 TI Multiple tissue transformation in adult zebrafish by gene gun bombardment and muscular injection of naked DNA.
 AU Sudha, Puttur Mudumana; Low, Sharon; Kwang, Jimmy; Gong, Zhiyuan (1)
 CS (1) Department of Biological Sciences, National University of Singapore, Singapore, 119260: dbsgzy@nus.edu.sg Singapore
 SO Marine Biotechnology (New York), (March April, 2001) Vol. 3, No. 2, pp. 119-125. print.
 ISSN: 1436-2228.
 DT Article
 LA English
 SL English
 AB The efficiency of two direct gene transfer methods, gene gun (or particle bombardment) and intramuscular injection, in transforming adult zebrafish tissues in vivo was examined by a noninvasive approach using green fluorescent protein (GFP) reporter gene driven by the ubiquitously expressed human cytomegalovirus promoter. Particle bombardment of adult zebrafish caused internalization and expression of the plasmid only in the superficial layer such as epithelial cells, pigment cells, endothelial cells, and neurons, whereas direct injection primarily transformed muscle fibers of several bundles near or around the injection site. Expression was also evident in several nonmuscle tissues, such as skin epithelia, pigment cells, blood vessel cells, and neuron-like cells. GFP expression persisted for more than 50 days with both methods. These observations indicate the potential of these methods for functional analysis of tissue-specific promoters, delivery of DNA vaccine, and muscular expression of other useful genes.

L35 ANSWER 3 OF 3 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 2
 AN 1998:78040 BIOSIS
 DN PREV199800078040
 TI Protection of goldfish against Ichthyophthirius multifiliis by immunization with a recombinant vaccine.
 AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong Jin; Sin, Yoke Min (1)
 CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge Crescent, Singapore 119260 Singapore
 SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
 ISSN: 0044-8486.

DT Article
LA English
AB A 316 bp gene fragment containing a potential antigenic epitope of the 48 kDa immobilization antigen of *Ichthyophthirius multifiliis* (i-AgI) was assembled from six synthetic oligonucleotides and cloned into a bacterial expression vector pGEX2T. The gene construct was introduced into *Escherichia coli* and the glutathione S-transferase-iAgI (GST-iAgI) fusion protein was successfully expressed. Antisera against GST-iAgI fusion protein from catfish showed a positive reaction with a tomite protein of about 48 kDa, suggesting that the recombinant protein contains an antigenic epitope of i-AgI. Naive goldfish that were immunized with purified GST-iAgI fusion protein were challenged with a lethal dose of infectious tomite of *I. multifiliis*. The results showed that the average survival rate of the immunized fish was 95% as compared to 55% for the control fish. All these findings suggest that the recombinant GST-iAgI fusion protein can be used as a potential vaccine against the infection of *I. multifiliis*.

=> d clm 1

L35 ANSWER 1 OF 3 USPATFULL

CLM What is claimed is:

1. An isolated skin-type intracellular antifreeze polypeptide, wherein

the polypeptide comprises an N terminal Met-Asp-Ala-Pro (SEQ ID NO:1)

subsequence; the polypeptide comprises an internal Ala-Ala-Thr-Ala-Ala-

Ala-Ala-Lys-Ala-Ala-Ala (SEQ ID NO:2) subsequence; the polypeptide does

not comprise a signal sequence; the polypeptide induces a concentration-dependent decrease in the freezing point of an aqueous

solution; and, conservative modifications thereof.

2. The isolated polypeptide of claim 1, wherein the polypeptide has a molecular of about 3400 Da.

3. The isolated polypeptide of claim 1, wherein the polypeptide has an N terminal Met-Asp-Ala-Pro-Ala (SEQ ID NO:9) sequence.

4. The isolated polypeptide of claim 1, wherein the polypeptide is from about 35 to about 55 amino acids in length.

5. The isolated polypeptide of claim 1, wherein the polypeptide comprises the sequence Met-Asp-Ala-Pro-Ala-X.sub.1 -Ala-Ala-Ala-Ala-Thr-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala-Glu-Ala-Thr-X.sub.2 -Ala-Ala-Ala-Ala-X.sub.2 -Ala-Ala-Ala-X.sub.3 -Thr (SEQ ID NO:3); wherein, X.sub.1 is selected from the group consisting of Arg, Lys, and Ala; X.sub.2 is selected from the group consisting of Lys and Ala; and, X.sub.3 is selected from the group consisting of Ala and Asp and a bond.

6. The isolated polypeptide of claim 1, wherein the polypeptide is selected from the group consisting of sAFP1 (SEQ ID NO:16), sAFP2 (SEQ ID NO:18), sAFP3 (SEQ ID NO:20), sAFP4 (SEQ ID NO:22), sAFP5 (SEQ ID NO:24), sAFP6 (SEQ ID NO:26), sAFP7 (SEQ ID NO:28), sAFP8 (SEQ ID NO:30), and 11-3 (SEQ ID NO:32).

7. The isolated polypeptide of claim 1, wherein the polypeptide binds to a pool of subtracted polyclonal antibodies, wherein the subtracted polyclonal antibodies are raised against the sAFP1 (SEQ ID NO:16) polypeptide and subtracted within HPLC-6 polypeptide (SEQ ID NO:39).

8. The isolated polypeptide of claim 1, wherein the isolated polypeptide is a component of an aqueous solution.

9. The isolated polypeptide of claim 1, wherein the polypeptide is from about 60% to about 70% helical as measured by circular dichroism.

10. The isolated polypeptide of claim 1, wherein the polypeptide is a fusion protein.

11. The isolated skin-type intracellular antifreeze polypeptide of claim 1, which is encoded by a nucleic acid molecule, which nucleic acid molecule hybridizes to a skin type antifreeze nucleic acid molecule selected from the group consisting of sAFP1 (SEQ ID NO:15), sAFP2 (SEQ ID NO:17), sAFP3 (SEQ ID NO:19), sAFP4 (SEQ ID NO:21), sAFP5 (SEQ ID NO:23), sAFP6 (SEQ ID NO:25), sAFP7 (SEQ ID NO:27), sAFP8 (SEQ ID NO:29), F2 (SEQ ID NO:33) and 11-3 (SEQ ID NO:31) in a northern blot

under high stringency wash conditions of 0.015M NaCl at 72.degree. C.,
wherein the nucleic acid molecule does not hybridize to SEQ ID NO:41
under high stringency wash conditions of 0.015NaCl at 72.degree. C.

12. The isolated polypeptide of claim 11, wherein the polypeptide is
selected from the group consisting of sAFP1 (SEQ ID NO:16), sAFP2 (SEQ ID NO:18), sAFP3 (SEQ ID NO:20), sAFP4 (SEQ ID NO:22), sAFP5 (SEQ ID NO:24), sAFP6 (SEQ ID NO:26), sAFP7 (SEQ ID NO:28), sAFP8 (SEQ ID NO:30), and 11-3 (SEQ ID NO:32).

13. A method of making an aqueous composition resistant to freezing,
comprising adding a skin-type antifreeze polypeptide to the composition
in an amount sufficient to change the thermal hysteresis of the composition, wherein the skin-type antifreeze polypeptide comprises an N terminal Met-Asp-Ala-Pro (SEQ ID NO:1) subsequence, and an internal Ala-Ala-Thr-Ala-Ala-Ala-Ala-Lys-Ala-Ala-Ala (SEQ ID NO:2) subsequence;
and wherein the polypeptide does not comprise a signal sequence.

14. The method of claim 13, wherein the step of adding the skin type antifreeze peptide is performed in a cell, wherein the skin type antifreeze polypeptide is added to the cell by transforming the cell with a nucleic acid which encodes the skin type antifreeze polypeptide
and expressing the antifreeze polypeptide in the cell.

=> s ichthyophthirius and vaccin?

L36 59 ICHTHYOPHTHIRIUS AND VACCIN?

=> s l36 and (recombin? or DNA or fusion)

L37 25 L36 AND (RECOMBIN? OR DNA OR FUSION)

=> dup rem l37

PROCESSING COMPLETED FOR L37

L38 13 DUP REM L37 (12 DUPLICATES REMOVED)

=> d bib ab 1-13

L38 ANSWER 1 OF 13 USPATFULL
 AN 2001:105334 USPATFULL
 TI DELIVERY OF NUCLEIC ACID INTO AQUATIC ANIMALS
 IN POET, STEVEN E., WINTERVILLE, GA, United States
 BURNLEY, VICTORIA VAUGHN, ATHENS, GA, United States
 PI US 2001006953 A1 20010705
 AI US 1999-347959 A1 19990706 (9)
 PRAI US 1998-91820 19980706 (60)
 DT Utility
 FS APPLICATION
 LREP SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.O. BOX 2938,
 MINNEAPOLIS, MN,
 55402
 CLMN Number of Claims: 48
 ECL Exemplary Claim: 1
 DRWN No Drawings
 LN.CNT 1265
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.
 AB Disclosed are methods for delivering a preselected polypeptide
 into an
 aquatic animal by contacting the aquatic animal with an
 aqueous medium
 containing an isolated non-infectious, non-integrating
 polynucleotide
 encoding an immunogen, wherein the polynucleotide is operably
 linked to
 a promoter that controls the expression of the polynucleotide
 in the
 aquatic animal, and wherein expression of the polypeptide
 stimulates a
 detectable biological response in the animal. Also disclosed
 are methods
 for delivering a desired polynucleotide into an aquatic animal
 comprising contacting the aquatic animal with an aquatic medium
 containing an isolated non-infectious, non-integrating
 polynucleotide,
 wherein the polynucleotide is substantially complementary to
 all or a
 portion of a messenger RNA (mRNA) encoding a preselected
 polypeptide,
 and wherein expression of the polypeptide stimulates or
 represses a
 detectable biological response in the animal. Methods are
 further
 disclosed for delivering a preselected polynucleotide into an
 aquatic
 animal comprising contacting the aquatic animal with an
 aqueous medium
 containing an isolated non-infectious, non-integrating
 polynucleotide
 that is not in contact with a liposome or lipid carrier,
 wherein the
 polynucleotide stimulates a detectable biological response in
 the
 animal.

L38 ANSWER 2 OF 13 USPATFULL
 AN 2001:55947 USPATFULL
 TI Methods and products for stimulating the immune system using

immunotherapeutic oligonucleotides and cytokines
IN Krieg, Arthur M., Iowa City, IA, United States
Weiner, George, Iowa City, IA, United States
PA University of Iowa Research Foundation, Iowa City, IA, United
States
(U.S. corporation)
PI US 6218371 B1 20010417
AI US 1999-286098 19990402 (9)
PRAI US 1998-80729 19980403 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Yucel, Remy; Assistant Examiner: Zara, Jane
LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims: 23
ECL Exemplary Claim: 1
DRWN 11 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 2746

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to synergistic combinations of
immunostimulatory CpG oligonucleotides and immunopotentiating
cytokines.

In particular, the invention relates to methods of stimulating
an immune

response using the synergistic combination of compounds and
products

related thereto.

L38 ANSWER 3 OF 13 USPATFULL

AN 2001:14470 USPATFULL

TI **DNA based vaccination** of fish

IN Davis, Heather L., Ottawa, Canada

PA Loeb Health Research Institute at The Ottawa Hospital, Ottawa,
Canada

(non-U.S. corporation)

PI US 6180614 B1 20010130

AI US 1998-115423 19980714 (9)

RLI Continuation of Ser. No. US 1996-740805, filed on 4 Nov 1996,
now

patented, Pat. No. US 5780448, issued on 14 Jul 1998

PRAI US 1995-6290 19951107 (60)

DT Utility

FS Granted

EXNAM Primary Examiner: Salimi, Ali R.

LREP Yankwich, Leon R., O'Brien, David G.

CLMN Number of Claims: 84

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 1280

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of immunization of
aquaculture

species by introducing **DNA** expression systems into the
aquaculture species. Such **DNA** expression systems preferably
include **DNA** sequences encoding polypeptides of pathogens of
species of aquaculture. The present invention also relates to
methods of

administration of **DNA** expression systems into aquaculture.

Such methods include injection, spray, and immersion
techniques. The

methods of this invention are useful for prophylactic **vaccination** or therapeutic immunization of fin-fish, shellfish, or other aquatic animals against infectious diseases.

L38 ANSWER 4 OF 13 EMBASE COPYRIGHT 2001 ELSEVIER SCI.

B.V.DUPLICATE 1

AN 2001299812 EMBASE

TI Host responses against the fish parasitizing ciliate **Ichthyophthirius** multifiliis.

AU Buchmann K.; Sigh J.; Nielsen C.V.; Dalgaard M.

CS K. Buchmann, Dept. of Veterinary Microbiology, Section of Fish Diseases,

Royal Veterinary and Agric. Univ., 4 Stigbojlen, DK-1870 Frederiksberg C,

Denmark. kurt.buchmann@vetmi.kvl.dk

SO Veterinary Parasitology, (12 Sep 2001) 100/1-2 (105-116).

Refs: 71

ISSN: 0304-4017 CODEN: VPARDI

PUI S 0304-4017(01)00487-3

CY Netherlands

DT Journal; Conference Article

FS 004 Microbiology

026 Immunology, Serology and Transplantation

037 Drug Literature Index

LA English

SL English

AB Recent studies have shown that fish are able to mount protective immune

responses against various parasites. One of the best characterized

parasite-host system in this context is the ciliate **Ichthyophthirius** multifiliis (Ich) parasitizing a range of freshwater fishes. Both specific and non-specific host defence mechanisms

are responsible for the protection of fish against challenge infections

with this ciliate. The specific humoral components comprise at least

specific antibodies. The non-specific humoral elements included are the

alternative complement pathway and probably lectins. Cellular factors

involved in the specific response are B-cells and putative T-cells. The

non-specific effector cells recognized are various leukocytes. In addition, goblet-cells and mast cells (EGC-cells) may have a function. The

NCC-cell (suggested analogue to NK-cells in mammals) seems to play a role

in the non-specific response. This well documented protective response in

freshwater fishes against Ich has urged the development of anti-parasitic

vaccines. Indeed, such products based on formalin killed parasites

have been developed and found to offer the **vaccinated** host a satisfactory protection. However, the collection of parasites for **vaccine** production is extremely laborious. It involves keeping infected fish due to the fact that in vitro propagation of the parasite is

still insufficiently developed. Gaining knowledge of amino acid sequences and its encoding **DNA**-sequences for the protective antigens (i-antigens) in the parasite was a major breakthrough. That achievement made it possible to produce a **recombinant** protein in *E. coli* and preliminary results indicated a certain protection of fish **vaccinated** with this product. Recent work has shown that the free-living and easily cultivated ciliate *Tetrahymena* can be transformed and express the i-antigen. This path seems to be promising for future development of **vaccines** against Ich. A novel approach in fish is the development of **DNA-vaccines**. Successful **DNA-vaccination** trials have been conducted in fish against viral infections and the technology also makes it possible to develop a **DNA-vaccine** against Ich. Other approaches to immuno-protection against Ich have been the use of heterologous **vaccines**. Thus, both bath and injection **vaccination** using live or killed (un-transformed) *Tetrahymena* has been reported to offer treated fish a certain level of protection. Such protection could be explained by non-specific reactions and the efficacy and duration of this **vaccination** type should be further evaluated. .COPYRG.T. 2001 Elsevier Science B.V. All rights reserved.

L38 ANSWER 5 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
 DUPLICATE
 2
 AN 2000-514962 [46] WPIDS
 CR 2000-506071 [44]
 DNN N2000-380572 DNC C2000-153683
 TI **Recombinant** expression systems for expressing heterologous nucleic acids and producing **recombinant** protein, comprises nonpathogenic protozoa such as *Tetrahymena* resistant to paclitaxel.
 DC B04 C06 D16 S03
 IN CLARK, T G; DICKERSON, H W; GAERTIG, J
 PA (CLAR-I) CLARK T G; (DICK-I) DICKERSON H W; (GAER-I) GAERTIG J; (UYGE-N)
 UNIV GEORGIA RES FOUND INC
 CYC 89
 PI WO 2000046381 A1 20000810 (200046)* EN 83p
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU
 MC MW NL
 OA PT SD SE SL SZ TZ UG ZW
 W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK
 DM EE ES
 FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
 LK LR LS
 LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG
 SI SK SL
 TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
 AU 2000027563 A 20000825 (200059)
 ADT WO 2000046381 A1 WO 2000-US2966 20000204; AU 2000027563 A AU
 2000-27563

20000204

FDT AU 2000027563 A Based on WO 200046381

PRAI US 1999-131121P 19990427; US 1999-118634P 19990204; US
1999-122372P

19990302; US 1999-124905P 19990317

AB WO 200046381 A UPAB: 20001117

NOVELTY - A **recombinant** protein expression system (I) comprises
a transgenic protozoan host cell resistant to paclitaxel
containing a

heterologous nucleic acid encoding a polypeptide, selectable by
negative

selection using paclitaxel.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included
for the

following:

(1) an expression vector (II) comprising a 5' flanking
region

followed by a heterologous nucleic acid encoding a polypeptide
comprising

at least one targeting amino acid sequence encoded by a portion
of an

i-antigen-encoding nucleotide sequence from **Ichthyophthirius**
multifiliis followed by a 3' flanking region, where at least a
portion of

each of the 5' flanking region and the 3' flanking region is
complementary

to an endogenous gene of an intended host, to allow integration
into the

endogenous gene by homologous **recombination**;

(2) a transgenic *Tetrahymena thermophila* comprising at
least a

portion of an *I. multifiliis* i-antigen protein;

(3) a transgenic cell (III) comprising a heterologous
protein

comprising at least one targeting amino acid sequence encoded by
an

i-antigen encoding nucleotide sequence from *I. multifiliis*;

(4) a method for preparing a polyclonal antibody;

(5) a method for detecting antibodies to an antigenic
polypeptide

comprising expressing the antigenic polypeptide on the surface
of a

transgenic protozoan host cell, exposing the host cell to an
antibody and

determining whether the swimming behavior of host cell is
altered, where

an alteration in the swimming behavior of the host cell is
indicative of

binding of the presence of antibodies to the antigenic
polypeptide; and

(6) screening (IV) drugs for the ability to bind a
polypeptide

comprising expressing the polypeptide on the surface of a
transgenic

protozoan host cell, exposing the host cell to a drug and
determining

whether the swimming behavior of host cell is altered, where an
alteration

in the swimming behavior of the host cell is indicative of
binding of the

drug to the polypeptide;

(7) a **vaccine** (V) comprising a transgenic non-pathogenic immunogenic protozoan comprising a surface-displayed antigenic polypeptide; and

(8) **recombinant** methods of producing a polypeptide.

ACTIVITY - Protozoacide.

MECHANISM OF ACTION - **Vaccine**.

Tetrahymena thermophila cells were transformed with the entire

IAG48(G1) gene of I. multifiliis G1 which encodes the GPI anchored 48 kDa

i-antigen or a truncated gene sequence. Transformants encoding the intact

or C-terminal truncated i-antigen were grown in standard Tetrahymena

growth medium. Groups of channel catfish were immunized intraperitoneally

with T. thermophila transformants producing intact or truncated i-antigen.

Fish were injected two times at a 30 day interval and challenged 21 days

after the last immunization G5 **Ichthyophthirius**. The results showed that a greater degree of protection was elicited in immunized fish

compared to controls. The antibody response of fish injected with live

cells secreting **recombinant** i-antigen was two fold greater than the antibody response of fish immunized with Tetrahymena producing the

membrane-bound intact i-antigen.

USE - The protein expression systems are useful for producing a

polypeptide, comprising introducing (I) into a protozoan host cell that is

resistant to paclitaxel, or a ciliated protozoan host cell to yield a

transgenic protozoan host cell that is selectable by negative selection

using paclitaxel and expressing the transgenic polypeptide in the transgenic protozoan host cell (claimed). The polypeptide is preferably an

antigenic polypeptide and is expressed on the plasma membrane surface of

the host cell and cleaved from the membrane surface of the transgenic host

cell. Transgenic ciliated protozoa are useful as live **vaccines** for stimulating an immune response in a vertebrate. The

transgenic protozoan host cells are useful for producing polyclonal antibodies

(claimed). The cells are also useful for detecting antibodies to the

antigenic polypeptide, by exposing host cells expressing the antigenic

polypeptide on the surface to an antibody and determining alteration in

the swimming behavior of the protozoan host cell, where swimming behavior

of the cell is altered in the presence of the antibodies to the antigenic

polypeptide. The host cell is immobilized and exposed to the body fluid of the patient suspected of being infected with the parasite (all claimed).

Tetrahymena expressing I.multifiliis i-antigen protein on their surface are effective vehicles for **vaccination** of freshwater fish against infection by I.multifiliis.

ADVANTAGE - The protein expression systems are suitable for large scale and analytical scale production of **recombinant** polypeptides and are particularly useful for expression of polypeptides that are difficult to produce in conventional **recombinant** protein expression systems.
Dwg.0/10

L38 ANSWER 6 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
DUPLICATE

3
AN 2000-506071 [45] WPIDS
CR 2000-514962 [44]
DNC C2000-151959
TI Novel i-antigen polypeptides and polynucleotides from
Ichthyophthirius multifiliis, useful for prophylaxis and
treatment
of **Ichthyophthirius** infection in fish.
DC B04 C06 D16
IN CLARK, T G; DICKERSON, H W; LIN, T
PA (CLAR-I) CLARK T G; (CORR) CORNELL RES FOUND INC; (DICK-I)
DICKERSON H W;
(LINT-I) LIN T; (UYGE-N) UNIV GEORGIA RES FOUND INC
CYC 89
PI WO 2000046373 A1 20000810 (200045)* EN 144p
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU
MC MW NL
OA PT SD SE SL SZ TZ UG ZW
W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK
DM EE ES
FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC
LK LR LS
LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG
SI SK SL
TJ TM TR TT TZ UA UG UZ VN YU ZA ZW
AU 2000027561 A 20000825 (200059)
ADT WO 2000046373 A1 WO 2000-US2962 20000204; AU 2000027561 A AU
2000-27561
20000204
FDT AU 2000027561 A Based on WO 200046373
PRAI US 1999-131121P 19990427; US 1999-118634P 19990204; US
1999-122372P
19990302; US 1999-124905P 19990317
AB WO 200046373 A UPAB: 20001117
NOVELTY - An i-antigen polypeptide having a defined sequence of
442 or 468
amino acids (given in the specification) from a G1 or G5 isolate
of
Ichthyophthirius multifiliis (or its fragment comprising
C-terminal or at least 1 terminal portion, its analog or an
antigenic

fragment), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid molecule (II) comprising a polynucleotide fragment having a nucleotide that encodes (I);
(2) a nucleic acid molecule that is complementary to (II);
(3) a composition (III) for inducing an immune response in a fish comprising (I) or (II);
(4) a host cell transformed with (II);
(5) a fish comprising (II);
(6) transformed Tetrahymena comprising (II);
(7) an antibody capable of binding (I);
(8) identifying an *Ichthyophthirius multifiliis* serotype comprising providing a sample comprising a *Ichthyophthirius multifiliis* nucleic acid molecule having a nucleotide sequence encoding an i-antigen, adding to the sample at least 1 primer oligonucleotide having a sequence complementary to a unique region of the *Ichthyophthirius multifiliis* nucleotide sequence, and subjecting the sample to amplification conditions; and
(9) detecting (IV) *Ichthyophthirius* in an aquaculture, comprising obtaining a sample containing nucleic acid from an aqua culture fish or an aqua culture water, adding at least 1 primer oligonucleotide having a sequence complementary to a portion of a sequence of 1326 or 1404 base pairs (bp) (defined in the specification) to the nucleic acid sample, conducting a polymerase chain reaction (PCR) amplification with the sample.

ACTIVITY - Protozoacide.

MECHANISM OF ACTION - Vaccine.

Effect of *I. multifiliis* i-antigens to elicit protective immune response in channel catfish was studied. Fish were immunized by two intraperitoneal injections consisting of 10 µg of purified 55 kD (kilodalton) i-antigen of the *I. multifiliis* G5 isolate in Freund's complete or incomplete adjuvant. As a positive control fish were vaccinated by two injections live, G5 parasites without adjuvant. All groups were challenged with infective G5 theronts 8 weeks after the last injection. Seventy-two percent of fish immunized with the i-antigen and 59.2% of fish immunized with live parasites survived the challenge while all of the negative control animals were dead.

USE - (III) is useful for prophylaxis, treatment or for controlling

I. multifiliis infection in fish. Polynucleotide or protein vaccine comprising a portion of the amplified product encoding an

antigenic polypeptide obtained by (IV) is useful for treating or preventing I. multifiliis infection in fish (claimed).

Dwg.0/21

L38 ANSWER 7 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-13144 BIOTECHDS
TI **Recombinant** expression systems for expressing heterogonous nucleic acids and producing **recombinant** protein such as Tetrahymena resistant to paclitaxel; vector-mediated **Ichthyophthirius** multifiliis gene transfer and expression in Tetrahymena thermophila for **vaccine** production
AU Gaertig J; Dickerson Jr H W; Clark T G
PA Univ.Georgia-Res.Found.
LO Athens, GA, USA.
PI WO 2000046381 10 Aug 2000
AI WO 2000-US2966 4 Feb 2000
PRAI US 990131121 27 Apr 1999; US 1999-118634 4 Feb 1999
DT Patent
LA English
OS WPI: 2000-514962 [46]
AB A **recombinant** protein expression system containing a transgenic protozoan host cell resistant to paclitaxel containing a heterologous nucleic cid encoding a protein, selectable by negative selection using paclitaxel. Also claimed are: an expression vector containing a 5' flanking region followed by a heterologous nucleic acid encoding a protein containing at least one targeting protein sequence encoded by a portion of an I-antigen nucleotide sequence from **Ichthyophthirius** multifiliis followed by a 3' flanking region, to allow integration into the endogenous gene by homologous **recombination**; a transgenic Tetrahymena thermophila containing a portion of an I. multifiliis I-antigen protein; a transgenic cell; preparing polyclonal antibodies; detecting antibodies; screening drugs for the ability to bind a protein; a **vaccine** of a transgenic non-pathogenic immunogenic protozoan containing a surface-displayed antigenic protein; and producing a protein. The protein expression system is used for producing an antigenic protein for expression on the surface of a host cell for **vaccine** production. (83pp)

L38 ANSWER 8 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION LTD
AN 2000-13109 BIOTECHDS
TI Novel i-antigen polypeptides and polynucleotides from **Ichthyophthirius** multifiliis, useful for prophylaxis and treatment of **Ichthyophthirius** infection in fish; plasmid pProEX-1-mediated gene transfer and expression in Escherichia coli, Pichia pastoris or Tetrahymena sp. and antibody for

**recombinant vaccine or nucleic acid vaccine
for gene therapy**

AU Clark T G; Dickerson Jr H W; Lin T L
PA Univ.Georgia-Res.Found.; Cornell-Res.Found; Clark T G;
Dickerson Jr H W;
Lin T L
LO Athens, GA, USA; Ithaca, NY, USA.
PI WO 2000046373 10 Aug 2000
AI WO 2000-US2962 4 Feb 2000
PRAI US 1999-131121 27 Apr 1999; US 1999-118634 4 Feb 1999
DT Patent
LA English
OS WPI: 2000-506071 [45]
AB An i-antigen protein having a defined 422 or 468 amino acid
protein
sequence from a GI or G5 isolate of **Ichthyophthirius**
multifiliis, is new. Also claimed are: a nucleic acid molecule
containing a polynucleotide encoding the protein; a
complementary nucleic
acid; a composition for inducing an immune response in a fish;
a host
cell (e.g. Escherichia coli, Pichia pastoris or Tetrahymena
sp.); a fish
containing the nucleic acid molecule; transformed Tetrahymena
containing
the nucleic acid molecule; an antibody; identifying an I.
multifiliis
nucleic acid encoding an i-antigen using a DNA primer; and
detecting **Ichthyophthirius** sp. in an aquiculture using a
DNA primer. Also disclosed are **vaccines** for preventing
diseases in fish caused by I. multifiliis and monoclonal or
polyclonal
antibodies. The composition is useful for prophylaxis,
treatment or for
controlling I. multifiliis infection in fish. A polynucleotide
or
protein **vaccine** containing an antigenic protein obtained by the
detection method is useful for treating or preventing I.
multifiliis
infection in fish. In an example, plasmid pProEX-1 was used to
transform
Escherichia coli. (144pp)

L38 ANSWER 9 OF 13 USPATFULL
AN 1998:82736 USPATFULL
TI DNA-based **vaccination** of fish
IN Davis, Heather L., Ottawa, Canada
PA Ottawa Civic Hospital Loeb Research, Ottawa, Canada (non-U.S.
corporation)
PI US 5780448 19980714
AI US 1996-740805 19961104 (8)
PRAI US 1995-6290 19951107 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Mosher, Mary E.; Assistant Examiner: Salimi,
Ali R.
LREP Fish & Richardson, P.C.
CLMN Number of Claims: 83
ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 1309

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention relates to methods of immunization of aquaculture

species by introducing **DNA** expression systems into the aquaculture species. Such **DNA** expression systems preferably include **DNA** sequences encoding polypeptides of pathogens of species of aquaculture. The present invention also relates to methods of

administration of **DNA** expression systems into aquaculture. Such methods include injection, spray, and immersion techniques. The

methods of this invention are useful for prophylactic **vaccination** or therapeutic immunization of fin-fish, shellfish, or other aquatic animals against infectious diseases.

L38 ANSWER 10 OF 13 WPIDS COPYRIGHT 2001 DERWENT INFORMATION LTD
DUPLICATE

4

AN 1997-282877 [26] WPIDS

CR 1998-242678 [22]

DNC C1997-091054

TI **DNA vaccines** for aquatic sp., e.g. carp, trout and oysters - containing **DNA** sequence encoding immunogenic polypeptide.

DC B04 C06 D16

IN DAVIS, H L

PA (OTTA-N) OTTAWA CIVIC HOSPITAL LOEB RES INST; (OTTA-N) OTTAWA CIVIC

HOSPITAL; (LOEB-N) LOEB RES INST OTTAWA CIVIC HOSPITAL; (LOEB-N) LOEB.

HEALTH RES INST AT OTTAWA HOSPITAL

CYC 9

PI EP 773295 A2 19970514 (199726)* EN 16p

R: DK FI FR GB SE

NO 9604713 A 19970509 (199729)

CA 2189831 A 19970508 (199736)

JP 09285291 A 19971104 (199803) 17p

US 5780448 A 19980714 (199835)

US 6180614 B1 20010130 (200108)

ADT EP 773295 A2 EP 1996-117859 19961107; NO 9604713 A NO 1996-4713 19961107;

CA 2189831 A CA 1996-2189831 19961107; JP 09285291 A JP 1996-295565

19961107; US 5780448 A Provisional US 1995-6290P 19951107, US 1996-740805

19961104; US 6180614 B1 Provisional US 1995-6290P 19951107, Cont of US

1996-740805 19961104, US 1998-115423 19980714

FDT US 6180614 B1 Cont of US 5780448

PRAI US 1996-740805 19961104; US 1995-6290P 19951107; US 1998-115423

19980714

AB EP 773295 A UPAB: 20010207

DNA vaccine for aquaculture species comprises an expression control sequence capable of directing expression of at least 1

immunogenic polypeptide and a polypeptide-encoding **DNA** sequence

encoding at least 1 immunogenic polypeptide from the genome of a pathogen.

Also claimed are: (1) a pharmaceutical composition (I) comprising a

DNA vaccine as above and a carrier; (2) a kit comprising (I) and at least 1 additional **DNA** expression vector comprising an expression control sequence capable of directing expression of at least

1 polypeptide and a polypeptide-encoding **DNA** sequence capable of facilitating an immune response for simultaneous, separate or sequential

use in immunising an aquaculture species; (3) a kit comprising (I) and an

adjuvant for separate or sequential use in immunising an aquaculture

species; (4) a kit comprising (I) and a **recombinant** protein for separate or sequential use in immunising an aquaculture species to boost

an immune response, and (5) a pharmaceutical composition for use in

expression of a polypeptide in an aquaculture species, which comprises a

DNA expression vector having an expression control sequence capable of directing the expression of at least 1 polypeptide and a

polypeptide-encoding **DNA** sequence encoding at least 1 polypeptide.

USE - (I) is useful for immunising an aquaculture species by i.m.

injection, i.p. injection, spray or immersion. The aquatic animal is

selected from salmon, salmonid, carp, catfish, trout (including rainbow

trout), yellowtail, sea-bream, sea-bass, eel, clams, lobster shrimp, crab

and oysters. The composition is designed for immunisation against pathogens selected from viral haemorrhagic septicaemia virus, infectious

haematopoietic necrosis virus, infectious pancreatic necrosis virus,

spring viraemia virus of carp, channel catfish virus (Herpesvirus ictaluri), grass carp haemorrhagic virus, nodaviridae such as nervous

necrosis virus or striped jack nervous necrosis virus, infectious salmon

anaemia, Aeromonis salmonicida, Renibacterium salmoninarum, Yersinia sp.,

Pasteurella sp., Vibrios sp., Edwardsiella sp., Streptococcus sp. and

Ichthyophthirius sp. (all claimed).
Dwg.0/0

L38 ANSWER 11 OF 13 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 5
AN 1998:78040 BIOSIS

DN PREV199800078040

TI Protection of goldfish against **Ichthyophthirius** multifiliis by immunization with a **recombinant vaccine**.

AU He, Jiangyan; Yin, Zhan; Xu, Guoliang; Gond, Zhiyuan; Lam, Toong
Jin; Sin,

Yoke Min (1)
 CS (1) Sch. Biol. Sci., Natl. Univ. Singapore, 10 Kent Ridge
 Crescent,
 Singapore 119260 Singapore
 SO Aquaculture, (Dec. 1, 1997) Vol. 158, No. 1-2, pp. 1-10.
 ISSN: 0044-8486.
 DT Article
 LA English
 AB A 316 bp gene fragment containing a potential antigenic epitope
 of the 48
 kDa immobilization antigen of *Ichthyophthirius multifiliis*
 (i-AgI) was assembled from six synthetic oligonucleotides and
 cloned into
 a bacterial expression vector pGEX2T. The gene construct was
 introduced
 into *Escherichia coli* and the glutathione S-transferase-iAgI
 (GST-iAgI)
 fusion protein was successfully expressed. Antisera against
 GST-iAgI fusion protein from catfish showed a positive reaction
 with a tomites protein of about 48 kDa, suggesting that the
 recombinant protein contains an antigenic epitope of i-AgI. Naive
 goldfish that were immunized with purified GST-iAgI fusion
 protein were challenged with a lethal dose of infectious tomites
 of *I.*
multifiliis. The results showed that the average survival rate
 of the
 immunized fish was 95% as compared to 55% for the control fish.
 All these
 findings suggest that the recombinant GST-iAgI fusion
 protein can be used as a potential vaccine against the infection
 of *I. multifiliis*.

L38 ANSWER 12 OF 13 BIOTECHDS COPYRIGHT 2001 DERWENT INFORMATION
 LTD
 AN 1996-04421 BIOTECHDS
 TI In vitro mutagenesis of an *Ichthyophthirius* surface antigen
 gene;
 point mutation introduction into immobilization antigen, for
 subsequent expression and use as a recombinant
 vaccine in fish (conference abstract)
 AU Dickerson H W; Clark T G
 CS Univ.Georgia
 LO Department of Medical Microbiology, College of Veterinary
 Medicine,
 University of Georgia, Athens, GA, USA.
 SO Conf.Res.Workers Anim.Dis.; (1995) 76 Meet., 163
 CODEN: 9999G
 Conference of Research Workers in Animal Diseases, 76th Annual
 Meeting,
 Chicago, IL, 13-14 November, 1995.
 DT Journal
 LA English
 AB The ciliate, *Ichthyophthirius multifiliis* (ICH), an important
 protozoan pathogen of freshwater fish. In the development of a
 subunit
 vaccine against ICH, a class of putative protective surface
 antigens called immobilization antigens (i-antigens), has been
 identified. Sequence analysis of cDNA and genomic DNA encoding
 the i-antigens reveals that ICH has an anomalous genetic code
 such that

commonly recognized stop codons, TAA and TAG, code for the amino acid glutamine instead. Thus, expression of ICH genes using conventional bacterial systems results in the production of truncated proteins. 8 TAA and TAG stop codons in the first 419 nucleotides of the cDNA encoding the 48 kDa antigen of ICH strain G1 were altered using polymerase chain reaction-based mutagenesis and overlap extension, and they subsequently read as glutamine codons CAA and CAG, respectively, when cloned into bacterial cells for expression. A 429 bp product is currently being sequenced to confirm presence of the 8 point mutations. The mutated product will be cloned into the plasmid vector pFLAG for ultimate large scale expression of the recombinant protein antigen. (0 ref)

L38 ANSWER 13 OF 13 MEDLINE DUPLICATE 6
AN 94122710 MEDLINE
DN 94122710 PubMed ID: 8293000
TI Serotypic variation among isolates of *Ichthyophthirius multifiliis* based on immobilization.
AU Dickerson H W; Clark T G; Leff A A
CS Department of Medical Microbiology, College of Veterinary Medicine,
University of Georgia, Athens 30602.
SO JOURNAL OF EUKARYOTIC MICROBIOLOGY, (1993 Nov-Dec) 40 (6)
816-20.
Journal code: BQT; 9306405. ISSN: 1066-5234.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199402
ED Entered STN: 19940314
Last Updated on STN: 20000327
Entered Medline: 19940225
AB Efforts have been made to determine whether surface antigens could be used as biochemical markers to define strain differences in the parasitic ciliate *Ichthyophthirius multifiliis*. In previous studies, a wild-type isolate designated G1 was found to have surface proteins analogous to the immobilization antigens of *Paramecium* and *Tetrahymena*; rabbit antiserum against this strain immobilizes homologous cells in vitro. It has now been shown for two additional *Ichthyophthirius* isolates (designated G1.1 and G2) that immobilization antigens are both present and serologically distinct. Proteins of similar size, which cross-react in Western blots with rabbit antisera against immobilization

antigens of the G1 strain, are nevertheless found in the G1.1 and G2 isolates. As shown by Southern blotting analysis, the G1.1 and G2 strains also contain genomic **DNA** sequences which hybridize with an immobilization antigen cDNA from G1 when probed under conditions of reduced stringency. The serotypic differences in immobilization between I. multifiliis isolates appear to be stable over time and provide a means of discriminating strains. In addition to providing a basis for comparative studies, the work described here has implications for the development of **vaccines** against this important fish parasite.